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(54) Title: PROTECTIVE EFFECTS OF MUTATED SUPERANTIGENS

(57) Abstract

The present invention includes a method for preventing and treating the toxic effects of a superantigen and for modifying pathogenic T cell responses in disease. Superantigen molecules are modified or mutated so that they no longer have the pathological effects of a superantigen, but are capable of eliciting an antibody response which crossreacts with and protects against the native superantigen. The molecules are useful, for example, as a vaccine. Mutated or modified superantigens that continue to interact with specific TCR Vβ-expressing subsets of T cells are also used to modify the target T cell population in a Vβ-specific manner.

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PROTECTIVE EFFECTS OF MUTATED SUPERANTIGENS

RELATED APPLICATIONS

This application is a continuation-in-part of USSN 08/242,694, filed May 13, 1994, entitled: Method for Modifying T Cell Response, a continuation of USSN 07/827,540, filed January 28, 1992, entitled: Method for Modifying T Cell Response, now abandoned; and PCT application PCT/US93/00839, filed January 28, 1993, entitled: Protective Effects of Mutated Superantigens.

FIELD OF THE INVENTION

This invention relates to methods for preventing and treating antigen-mediated and antigen-initiated diseases. Specifically, it relates to providing protection against superantigens of pathogens by the administration of modified or mutated superantigen molecules in order to elicit an antibody response against the superantigen without the pathological effect of the superantigen. The molecules of this invention may also interact with the V_β elements of T cell receptors in a way that leads to modifications in the way T cells respond to an antigen.

BACKGROUND OF THE INVENTION

The vertebrate immune system evolved to protect vertebrates from infection by microorganisms and large parasites. The immune system responds to antigens in one of two ways: (1) humoral antibody responses, mediated through B lymphocytes, or B cells, involving the production of protein antibodies that circulate in the bloodstream and bind specifically to the foreign antigen that induced them. The binding of the antibody to the antigen makes it easier for phagocytic cells to ingest the antigen and often activates a

system of blood proteins, collectively called complement, that helps destroy or clear the antigen; and (2) cell-mediated immune responses, mediated through T lymphocytes, or T cells, involving the production of specialized cells that react mainly with foreign antigens on the surface of host cells, either killing the host cell if the antigen is an infecting virus or inducing other host cells, such as macrophages, to destroy the antigen (Molecular Biology of the Cell (1983), B. Alberts et al. (eds), chapter 17, pp. 952).

The production of antibodies requires a number of conditions precedent in order to stimulate B cells into producing antibodies. One key event involved in the processes leading to antibody production is that of antigen recognition. Antigen recognition requires the participation of thymus (T) cells.

T cells have antigen-specific receptors on their surfaces, termed T cell antigen receptors (TCR). Before T cells can recognize protein antigens, the antigens must be presented on the surface of antigen-presenting cells. Macrophages or other antigen presenting cells must first process the antigens. These cells essentially swallow antigens and chop them into peptides which are displayed at the cell surface in combination with major histocompatibility complex (MHC) molecules (Shimonkevitz et al. (1983) J. Exp. Med. 158:303; Babbit, et al. (1985) Nature 317:359).

The major histocompatibility antigens are a family of molecules encoded by a related group of genes encoded within the major histocompatibility complex. MHC antigens are expressed on the cells of all higher vertebrates. In man they are called HLA antigens (human-leucocyte-associated antigens) because they were first demonstrated on leukocytes. There are two principal classes of MHC molecules, class I and class II, each

comprising a set f cell-surface glycoproteins. The two classes of MHC antigens stimulate different subpopulations of T cells. MHC class II molecules are involved in most responses to extracellular pathogens. In contrast, MHC class I molecules are involved when the pathogen is cell-associated, i.e., when a virus or a malignant cell is involved. When MHC class I is involved, antibody stimulation does not result; rather, the interaction of MHC class I processed antigen and T-cell leads to lysis of cells infected with the pathogen.

Processed antigen peptide fits in a cleft on an MHC molecule (Bjorkman et al. (1987) Nature 329:506). Once an antigen is displayed, the few T cells in the body that bear receptors for that particular peptide bind that complex. Most T cells recognize antigens on the surface of cells only in association with self-MHC glycoproteins expressed on the cell surface.

The ability of the T cell to bind to the processed antigen and MHC complex is dependent on the T cell receptor (TCR). The TCR consists of two protein chains, usually α and β chains. Each chain is composed of a constant and a variable domain. The variable domains are encoded in two (α) or three (β) different gene segments (variable (V), diversity (D), joining (J)) (Siu et al. (1984) Cell 37:393; Yanagi et al. (1985) Proc. Natl. Acad. Sci. USA 82:3430). In each T cell, the combination of V, D, and J domains of both the α and β chains participates in antigen recognition in a manner which is uniquely characteristic of that T cell and defines a unique binding site. (See, Marrack et al. (1988) Immunol. Today 9:308; Toyonaga et al. (1987) Ann. Rev. Immunol. 5:585; Kronenberg (1985) Ann. Rev. Immunol. 4:529; Hedrick et al. (1982) Cell 30:141.) Generally, both the α and β chains are involved in recognition of the ligand formed by processed antigen and MHC.

When T cells are stimul ted by an antigen, they divide and differentiate into activated effector cells that are responsible for various cell-mediated immune reactions. At least three different reactions are carried out by T cells: (1) cytolytic T cells specifically kill foreign or virus-infected vertebrate cells; (2) helper T cells help B lymphocytes; and (3) suppressor T cells suppress the responses of specific cells.

It has been shown that a novel class of antigens, termed "superantigens", are able to directly stimulate T cells by binding to a particular subset of the V_β element. That is, the variable domain of the β chain of the TCR (Kappler et al. (1987) Cell 49:263; Kappler et al. (1987) Cell 49:273; MacDonald et al. (1988) Nature 332:40; Pullen et al. (1988) Nature 335:796; Kappler et al. (1988) Nature 332:35; Abe et al. (1988) J. Immunol. 140:4132; White et al. (1989) Cell 56:27; Janeway et al. (1989) Immunol. Rev. 107:61; Bekoff et al. (1988) J. Immunol. 139:3189; Kappler et al. (1989) Science 244:811). Unlike recognition of conventional peptide antigens, the other components of the T cell receptor (i.e., D_β, J_β, V_α, J_α) appear to play little role in the superantigen binding. Superantigens, while generally stimulatory to T cells, appear to interact specifically with particular V_β elements present on the stimulated T cell. Since the relative number of V_β genes is limited, many T cells within an individual will bear a particular V_β element, and a given superantigen is therefore capable of interacting with a large fraction of the total T cell repertoire. Thus, depending on the frequency of the responding V_β population(s), 5-30% of the entire T cell repertoire could be stimulated by a superantigen, whereas the responding frequency to a conventional antigen is usually much less than 1 in 1,000, or .001%. Although superantigens interact with class II MHC molecules, they appear to act as intact proteins rather than

as peptides. That is, they do not require processing for stimulation and they bind outside the conventional peptide binding groove (Jardetzky et al. (1994) Nature 368:711; Seth et al. (1994) Nature 369:324). Instead, they appear to interact with amino acid residues that are on the outer walls of the binding cleft. Known superantigens and references to their sequences and structures are listed in Table I.

Two distinct classes of superantigen have been described. The first was noted over 20 years ago, when Festenstein showed marked responses in mixed lymphocyte reactions between certain MHC identical strains. The stimulating antigens were called minor lymphocyte stimulating (Mls) antigens (Festenstein (1973) Transplant Rev. 15:62) to differentiate them from MHC antigens. At that time, their superantigen character was not known. It is now known that these superantigens are encoded by endogenous retroviral genes (Palmer (1991) Curr. Bio. 1:74). The presence of these genes in the mouse leads to a marked deletion of responding T cells, creating potentially large holes in the animal's T cell receptor repertoire (Pullen et al. (1988) supra). The second class of superantigen is represented by a growing list of bacterial and viral proteins, capable of producing a variety of pathological effects after injection (Marrack & Kappler (1990) Science 248:705).

Staphylococcus aureus (S. aureus), a common human pathogen, produces several enterotoxins, designated as SEA (staphylococcal enterotoxin A) through SEE (staphylococcal enterotoxin E), which can be responsible for food poisoning and occasionally shock in humans (Marrack & Kappler (1990) supra; Bohach et al. (1990) Crit. Rev. Microbio. 117:251). Some S. aureus isolates also produce toxic shock syndrome toxin-1 (TSST-1), which has been implicated in the majority of cases of human toxic shock syndrome as well as

the related exfoliative toxins (ExT), which are associated with the scalded skin syndrome. Streptococcus pyrogenes, or group A streptococcus, another common human pathogen of the skin and pharynx, also produces toxins with superantigenic properties (Abe et al. (1991) J. Immun. 146:3747). These have been designated streptococcal erythrogenic toxins A-C (SPEA-C).

The amino acid sequence of the S. aureus toxins exhibit some homology, but also exhibit marked differences (See, Betley et al. (1988) J. Bacteriol. 170:34; Jones et al. (1986) J. Bacteriol. 166:29; Couch et al. (1988) J. Bacteriol. 170:2954; Blomster-Hautamaa et al. (1986) J. Biol. Chem. 261:15783). S. aureus toxins have the ability to stimulate powerful T cell proliferation responses in the presence of mouse cells bearing MHC class II type molecules (White et al. (1989) supra). The S. aureus proteins selectively stimulate murine cells bearing particular V_β elements.

The binding of toxins to class II MHC molecules is usually required for T cell recognition. However, the process is much more permissive for superantigens than that seen with conventional antigens. While peptide antigens are very dependent on allelic MHC residues for binding, the superantigens bind to a wide variety of allelic and isotypic forms of MHC class II molecules (See, Herrmann et al. (1989) Eur. J. Immunol. 19:2171; Herman et al. (1990) J. Exp. Med. 172:709; Scholl et al. (1990) J. Immunol. 144:226; Molleck et al. (1991) J. Immunol. 146:463). Whereas T cells rarely recognize peptide antigens bound to self-MHC (allo-MHC) molecules, individual T cell clones can respond to toxins bound not only to various allelic forms of MHC but also to different class II isotypes and even xenogeneic class II molecules. Such observations reinforce the concept that superantigens bind

at a relatively conserved site outside the allelically variable groove where conventional peptide antigens bind.

Superantigens may contribute to autoimmune diseases in which components of the immune system attack normal tissue. The process of deletion of T cells responsive to self, potentially harmful self-reactive T cells, is called tolerance or negative selection (Kappler et al. (1987) supra; Kappler et al. (1988) supra; Von Boehmer et al. (1988) Immunol. Rev. 101:21). The immune system usually deletes self-reactive T cells, with good, but not complete efficiency, but some self-reactive cells appear to escape the surveillance mechanism. It has been suggested that the ability of superantigens to arouse 20 percent of a person's T cell repertoire could lead to undesirable replication of the few circulating T cells that are capable of recognizing self (Kotzin et al. (1993) Adv. Immunol. 54:99). T cells bearing certain V_β types have been implicated in various autoimmune conditions, such as arthritis and multiple sclerosis. These destructive cells might have been initially activated by a superantigen that binds to the identified V_β types (Kotzin et al. (1993) supra), leading to disease.

Autoimmune diseases are a result of a failure of the immune system to avoid recognition of self. The attack by the immune system of host cells can result in a large number of disorders, including neural diseases such as multiple sclerosis and myasthenia gravis, diseases of the joints such as rheumatoid arthritis, attacks on nucleic acids as observed with systemic lupus erythematosus, and diseases associated with various organs such as psoriasis, juvenile onset diabetes, Sjögren's disease and thyroid disease.

Several lines of evidence suggest that T cells specific for self-antigens may play a critical role in the initiation of autoimmune diseases. In the case of

rheumatoid arthritis, the linkage of the disease to the DR4 and DR1 alleles of the class II genes of MHC, and the finding of oligoclonal activated $CD4^+$ T cells in synovial fluid and tissue of affected joints (Stastny et al. (1976) Engl. J. Med. 298:869; Gibofsky et al. (1978) J. Exp. Med. 148:1728; McMichael et al. (1977) Arth. Rheum. 20:1037; Schiff et al. (1982) Ann. Rheum. Dis. 41:403; Duquestoy et al. (1984) Hum. Immunol. 10:165; Legrand et al. (1984) Am. J. Hum. Genet. 36:690; Gregerse et al. (1987) Arth. Rheum. 32:15; Burmester et al. (1981) Arth. Rheum. 24:1370; Fox et al. (1982) J. Immunol. 128:351; Hemler et al. (1986) J. Clin. Invest. 78:696; Stamenkoic et al. (1988) Proc. Natl. Acad. Sci. USA 85:1179; Paliard et al. (1991) Science 253:325) suggest the involvement of $CD4^+$, $\alpha\beta$TCR-bearing, class II-restricted T cells in the disease. This view is supported by the finding that partial elimination or inhibition of T cells by a variety of techniques can lead to an amelioration of disease in certain patients (Paulus et al. (1977) Arth. Rheum. 20:1249; Karsh et al. (1979) Arth. Rheum. 22:1055; Kotzin et al. (1989) N. Eng. J. Med. 305:976; Herzog et al. (1987) Lancet ii:1461; Yocum et al. (1989) Ann. Int. Med. 109:863).

U. S. Patent No. 5,298,369, herein specifically incorporated by reference, establishes that specific V_β elements may be used to diagnose an autoimmune disease. Specifically, the presence of a higher percentage of $V_\beta 14^+$ T cells in synovial fluid may be used to diagnose rheumatoid arthritis.

Many investigative efforts have focused on developing methods for the treatment of autoimmune diseases. For example, European Patent Publication 340 109, entitled Anti-T-cell receptor determinants as autoimmune disease treatment, and U.S. Patent No. 4,550,086, issued October 29, 1985 to Reinherz et al., entitled Monoclonal antibodies that recognize human T cells,

describe a method of detecting a particular sequence of the variable region gene of T cell receptors associated with a particular disease and a treatment with antibodies to that sequence. U.S. Patent No. 4,886,743, issued December 12, 1989 to Hood et al., entitled: Diagnostic reagents based on unique sequences within the variable region of the T cell receptor and uses thereof, describes a method of diagnosing diseases based on the presence of T cells with a unique sequence in the V_β region associated with a specific disease. PCT Patent Application Publication WO 90/06758 describes a method for detecting specific V_β regions associated with RA, specifically, $V_\beta 3$, $V_\beta 9$, and $V_\beta 10$, and for the treatment of rheumatoid arthritis with monoclonal antibodies which recognize $V_\beta 3$, $V_\beta 9$, and $V_\beta 10$.

An animal that has never been exposed to a pathogen has no specific defenses against it. However, the animal can be immunized against the pathogen by injecting it with a non-virulent form of the pathogen similar in chemical structure to the pathogen but without the ability to cause the pathological effect. The animal will produce antibodies specific against the non-virulent form of the pathogen. These antibodies are able to protect the animal against attack from the virulent pathogen.

BRIEF SUMMARY OF THE INVENTION

One embodiment of the present invention includes the administration of a molecule in the form of a vaccine for preventing the toxic effects of a superantigen, wherein said molecule elicits antibody production to the superantigen without superantigen-mediated T cell stimulation. The molecule induces T cell stimulation but not in a superantigenic fashion.

The present invention also includes molecules consisting of mutated or modified derivatives of superantigens, wherein said superantigens are TSST-1 superantigens.

In an alternative embodiment of the present invention, the administration of a molecule that interacts with the V_β element of T cell receptors (TCR) acts to modify the T cell response elicited by an antigen.

The present invention also includes the treatment of a subject animal with a mutated or modified superantigen in order to affect said subject's T cell responsiveness.

The molecules of this invention can also function by leading to deletion or inactivation/desensitization of at least one or more subpopulations of T cells presenting a particular V_β element.

In order to prevent the *in vivo* toxic effect of a superantigen, it is required that the pathological condition mediated or initiated by a superantigen be able to be prevented or treated by administration of the mutant superantigen molecules of the present invention. Administration of the mutant toxins of the present invention may cause antibody production against the mutant molecule. Some of these antibodies also react with the normal non-mutated toxin. Therefore, when the immunized individual is confronted with

the path genic toxin, these cross-reactive antibodies react with the native toxin and inhibit its toxic activity.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 shows the nucleotide sequence of mature TSST-1 without the signal peptide cloned into pUC18 for the expression in E.coli. (SEQ ID NO:1) The figure also indicates oligonucleotide primers (A top primer (SEQ ID NO:2) and B bottom primer (SEQ ID NO:3)) used for the mutagenesis: dashed lines indicate A top primer and B bottom primer, respectively; solid lines indicate the sequences of mutagenic primers (SEQ ID NOS:4-16) used to generate the TSST-1 mutants by PCR.

FIGURE 2 shows the diagram for generation of TSST-1 mutants by the "megaprimer" PCR method.

FIGURE 3 shows the outline for isolation of TSST-1 mutants that produces a correctly folded TSST-1 (ELISA positive), yet lacks the ability to stimulate a mouse $V_\beta 15$-expressing T cell (T cell stimulation negative).

FIGURE 4 shows stimulation of T cells in human PBL by HPLC-purified wild-type and mutant rTSST-1 proteins.

FIGURE 5A shows the binding of TSST-1 and TSST-1 mutants to DR-bearing RAJI cells as detected by anti-TSST-1 monoclonal antibody. The single mutant rTSST-1 isolate that failed to demonstrate binding to HLA-DR on RAJI cells is the mutant G31S/S32P.

FIGURE 5B shows the direct binding of TSST-1 and the G31S/S32P TSST-1 mutant to DR-bearing RAJI cells. The G31S/S32P mutant protein binds HLA-DR at 4-5 log higher protein concentrations than can be demonstrated using the wild type toxin.

FIGURE 6 is a schematic ribbon drawing of the three dimensional structure of TSST-1. Residues identified by mutational analysis as important to MHC class II or TCR binding are indicated.

DETAILED DESCRIPTION OF THE INVENTION

The molecules of the present invention may be effective in different ways in preventing or treating antigen-mediated or initiated diseases. Some of the different ways in which the molecules of the present invention may be effective include modification of the T cell response and production of antibodies that provide protection against pathogens. Specifically, this invention presents a method of preventing or treating superantigen-mediated or superantigen initiated diseases. The method of this invention generally involves preparing mutated or modified superantigen molecules by methods known in the art and described herein, identifying antigen mutants unable to bind either MHC or TCR, and testing for the ability to protect against exposure to the non-mutated superantigen.

The present invention describes the feasibility of the above-outlined approach in achieving protection against a known superantigen. Mutants of recombinant *S. aureus* toxic shock syndrome toxin-1 (TSST-1) were prepared and purified as described in Examples 1-6 below. TSST-1 mutants unable to bind MHC molecules or TCR were selected by examining the binding of mutant TSST-1 molecules to HLA-DR positive lymphoblastoid line RAJI cells, stimulation of T cell hybridomas bearing different V_β elements, and stimulation of human peripheral blood T cells *in vitro*. Although the present disclosure describes production of mutated TSST-1 molecules able to protect subject animals against subsequent challenge with TSST-1, the methods of the present invention are equally applicable to other superantigens.

The ability to use the occurrence of specific V_β elements to diagnose autoimmune diseases, as discussed, may be combined with the present invention as a method of detecting, preventing and treating autoimmune

diseases mediated by superantigens. The existence of a superantigen-mediated disease may be determined by a "footprint" analysis, e.g., by determining if there is an alteration in V_β elements in a disease state. The finding of alterations in V_β elements, such as the increase in $V_\beta 14^+$ T cells in synovial fluid in rheumatoid arthritis, suggests the presence of a superantigen-mediated disease. Techniques known to the art may then be applied in order to isolate and identify the implicated superantigen. The V_β footprint may be compared against that of a known superantigen for possible implication of that superantigen in initiation or proliferation of the disease. There may be a search for genes coding for a superantigen when a virus or bacterial infection is associated with the initiation of the disease. Once a superantigen is identified or isolated, the method of the present invention may be applied to produce a mutant superantigen molecule capable of conferring protection against exposure to the superantigen.

Various terms are used in this specification, for which it may be helpful to have definitions. These are provided herein, and should be borne in mind when these terms are used in the following examples.

As described above, the key event in an immune response is the interaction of MHC molecules with antigens to form a complex presented to T cells. Generally, the T cell response is quite specific, with only very limited subpopulations of T cells responding to specific complexes of antigen and MHC molecules. The response generally requires interaction of most or all of the components of the T cell receptor. In certain circumstances, however, the presented antigen need only interact with the V_β element of the receptor, all other components being essentially inconsequential. That is, the proteins binding to all TCRs that utilize a particular V_β gene segment, regardless of the

other parts of the antigen receptor, are called superantigens. Superantigens are usually presented to T cells by class II MHC molecules, and T cell responses to superantigen-MHC complexes are apparently similar to responses to peptide-MHC complexes. This means that the superantigen can, and does, react with a much greater array of T cells than is normally the case.

The molecules of this invention may interact with the V_β elements of TCRs in a way which leads to modifications in the way T cells respond to a superantigen. "Modifying T cell responsiveness" means that the molecules are able to change the manner in which the subject's T cells respond when provoked by the administered molecule, or to an antigen administered prior to, concurrently, or subsequently. For example, it is believed that early in the development of T cells, certain subpopulations interact with presented self antigens and are deleted. The molecules of this invention can function in this manner, i.e., by leading to deletion or inactivation/desensitization of at least one or more subpopulations of T cells presenting a particular V_β element.

In a particular embodiment of the present invention, the molecules modify a subject animal's T cell response without changing the B cell response that would normally occur in the subject under consideration. The superantigen is provided in a form that does not function as a superantigen. That is, it does not stimulate T cells in a superantigenic way and elicits a specific T cell dependent antibody response. This type of material is useful, for example, for providing passive immunity to a subject or serving as a vaccine.

The molecules of the present invention may be regarded as competitors for other antigens at the TCR binding level. If the molecules described herein interact with MHC elements otherwise required for generation of a full scale

response to an antigen or superantigen, they may prevent or reduce the extent of that response.

Moreover, the molecules of the present invention may be viewed as "enhancers," for example, in cases in which an individual's T cell responsiveness is impaired or weakened by any of a number of causes. Via administration of the molecules encompassed by the present invention, the T cell populations of the individual can be greatly expanded.

The term "modifying T cell responsiveness" as used herein is always relative to a second element (e.g., an antigen), and always refers in particular to responsiveness of T cells presenting a particular V_β element as part of their T cell receptors, other components of the receptors being essentially irrelevant. The T cell response may be modified by maintaining the TCR binding site and modifying some other functional part, for example, the MHC binding site. Thus, effecting T cell specificity without stimulation, through the deletion of a T cell subset, anergy or blocking competitively at the T cell binding level. Alternatively, the TCR binding site may be modified (but not eliminated) in order to modify the T cell response. That is, maintaining the ability to interact with T cells while retaining T cell V_β selectivity, e.g., interacting with a different subpopulation.

"Reducing responsiveness" is construed to also include deleting the portion of T cells expressing a particular V_β element.

"Superantigen derivative" as used herein refers to a molecule whose structure, at the least, contains an amino acid sequence substantially identical to an amino acid sequence presented by a superantigen or portions of a superantigen required for binding to either the MHC or the T cell.

"Modified" superantigen derivative (or fragment), differs from "mutated" superantigen derivative (or fragment). The term "modified superantigen" is defined to refer to molecules which contain an amino acid sequence identical to a superantigen or portions of a superantigen, but contain modifications not found in the superantigen molecule itself. For example, if a superantigen contains amino acids 1-250, a "modified" superantigen derivative may contain a sequence identical to amino acids 50-75, positioned in between stretches of amino acids not found in the native superantigen molecule. Additional modifications may include, for example, differing or absent glycosylation patterns, or addition of other non-superantigen structures that modify function or distribution.

"Mutated" superantigen refer to structures where the actual amino acid sequence of the mutation has been altered relative to the native form of the molecule. For example, if a superantigen contains amino acids 1-250, a mutated superantigen may include amino acids 50-68 and 72-75 which are identical to the corresponding native sequence, but differ in amino acids 69-71. The difference may be one of "substitution" where different amino acids are used, "addition" where more amino acids are included so that the sequence is longer than the native form, or "deletion" where the amino acids are missing.

"Vaccine" refers to a formulation that when administered to a subject provokes the same type of response typical of vaccines in general, e.g., active immunological prophylaxis. The vaccine may contain adjuvant, or other biocompatible materials.

It is known that the class of molecules known as superantigens interact with particular V_β regions of TCRs, leading to massive proliferation of

particular T cell subpopulations. This interaction is mostly independent of any other region of the TCR. This interaction also usually involves prior interaction between an MHC molecule and the superantigen.

In connection with the interaction of MHC and peptide, it must be noted that class II MHC molecules are available in a variety of isotypes and allotypes, and particular molecules are specifically involved in the presentation of various peptides. In contrast, a superantigen usually can be presented by many different MHC molecules. Specifically, most HLA-DR molecules, regardless of allotypic differences are capable of presentation of TSST-1. Determination of HLA haplotype and correlation to presentation of a particular antigen is well within the skill of the artisan in this field.

This invention involves the modification of the T cell response via the administration to a subject of a molecule which interacts with an MHC molecule or at least one V_β element on the T cell receptor, or both. Said interaction may be accomplished through deletion/elimination of a T cell subpopulation, anergy, functional response modification (e.g., changing the cytokine profile) or blocking at the TCR level.

The modification of the T cell response may involve deletion of T cell subpopulations. Knowledge of the mechanisms described herein permits the artisan to administer to a subject a material which interacts with the MHC or a particular subpopulation of T cells, dependent on the TCR V_βs expressed, where activation and subsequent deletion of the target T cell subpopulation results. This approach is particularly desirable in the treatment of conditions where a particular V_β subpopulation or subpopulations are associated with a pathological condition, such as an autoimmune disease.

Another manner of modifying the T cell response is via "desensitizing", "inactivating", or "anergizing" the T cells. This mechanism involves interaction of the superantigen with target T cells via the TCR, with subsequent down regulation or inactivation of the T cells. Alterations in how the superantigen is presented to T cells may greatly increase this consequence of superantigen injection (Kotzin et al. (1993) supra) Furthermore, prior proliferation of the target T cells may not be discernable despite clear evidence of TCR binding of the superantigen by target T cells.

One aspect of the invention involves the use of mutated or modified superantigen molecules which have been altered so as not to induce a superantigen response but which still elicit a T cell dependent B cell response which is not different from that elicited by the native superantigen. Such materials are especially useful as vaccines, as discussed below.

The molecules of the invention are preferably, but not exclusively, superantigen derivatives. These derivatives may be modified or mutated, as discussed above. These, or any other molecules used herein, are administered in an amount sufficient to modify the T cell response in the manner described. The amount of material used will vary, depending on the actual material, the response desired, and the subject matter of the treatment.

The molecules may serve as vaccines. These vaccines confer protective immunity on the subject in that they generate a B cell response without the full superantigen-mediated T cell response normally associated with the native or wild-type form of the molecule. This effect has been previously shown using SEB (Tseng, et al. (1993) Inf. & Immun. 61:391). Again, depending upon the parameters within the control of the knowledge of the artisan, including the condition being treated, the V_β molecule to be

regulated, and so forth, the material chosen for the vaccine is up to the artisan. The vaccine may contain other materials which are normally found in vaccine compositions, including adjuvants, carriers, etc.

With regard to protection, superantigens generally function to activate a certain subpopulation of T cells. In certain circumstances, the expansion of a T cell population is followed by a subsequent deletion of that same subpopulation, or steady-state reduction of T cells of that subpopulation of T cells. In other cases the administration of a superantigen leads to a T cell subpopulation deletion without an intervening activation/expansion step. In these cases, the same subpopulation of T cells characterized by V_β element that would normally be expanded by the superantigen is the subpopulation that is deleted. Based on V_β element, there is a footprint that associates certain T cell populations to a given superantigen. According to this invention, it is possible that the mutated superantigens will be able to effect T cell responsiveness for T cell populations other than the populations that are a part of the native superantigen's footprint. Therefore, a library of mutated superantigens can be created in which it is possible to select which T cell populations are to be affected (with subsequent expansion, deletion or functional inactivation). It is unnecessary to know the actual superantigen associated with a given superantigen mediated disease in order to treat that disease. It is only necessary to know which subpopulation of T cells are involved in disease pathogenesis and therefore should be targeted.

The mode of administration of the materials described herein may include intravenous, intraperitoneal, and intramuscular injections, selective administration or targeting to regional arc, as well as all of the other standard methods for administering therapeutic agents to a subject.

The invention als discloses how to make particular mutants or modifications useful in the foregoing methodologies, including isolated nucleic acid sequences coding for mutants, cell lines transformed by these and the vectors and plasmids used therefor, as well as the isolated mutant molecules, including mutant superantigens.

Other applications of the invention described herein will be apparent to the skilled artisan and need not be repeated here.

The terms and expressions which have been employed are used as terms of description and not of limitation. There is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

Polymerase Chain Reaction (PCR) and standard molecular biological methodologies, described in Example 1, were used in the construction and expression of recombinant TSST-1. TSST-1 mutants were generated as described in Example 2. The method involves the introduction of random mutations along the entire length of the TSST-1 gene. Initial identification of potential mutant TSST-1s involved testing the lysate from transformants for the presence of functional toxin by stimulation of murine T cell hybridomas bearing $V_\beta 15$ elements after presentation by a human HLA-DR-expressing cell line. Lysates negative for T cell hybridoma stimulations were tested for the presence of TSST-1 protein levels with the use of monoclonal antibodies (mAbs) against TSST-1 (Example 3). Transformants producing non-functional TSST-1 were sequenced and the mutation identified. Transformants producing mutant TSST-1s were grown and mutant TSST-1s purified as described in Example 4. Analysis of the location and effect of the

mutation was performed. Mutants were tested for their ability to stimulate proliferation of human peripheral blood T cells as described in Figure 4. Binding to MHC class II molecules may be required for toxin recognition by T cells, the ability of mutant TSST-1 to bind human MHC antigen HLA-DR was also tested as described in Example 5. Thus, each mutation was classified as altering HLA-DR binding on human cells or interaction with human TCR.

As it is involved in superantigen-mediated T cell stimulation, the molecule of the instant invention holds dual functions: the administration as a vaccine and the modification of the T cell response of the subject. As a vaccine, the molecule is provided in a form that does not function as a superantigen. It does not stimulate T cells in a superantigenic way, but elicits a specific T cell dependent antibody response. Critical mutations for this type of activity include the T cell receptor site or the MHC binding site. However, there are data to suggest that the MHC binding site may not be essential for superantigen activity (Hamad et al. (1994) J. Exp. Med. 180:615). Said mutations cannot affect the 3-dimensional structure. The molecule must appear as much like a superantigen as possible so as not to alter its appearance to the antibodies. The second function is to effect a modification of the T cell response. This may be accomplished by maintaining the TCR binding site and modifying some other functional part or modifying the TCR binding site so that it interacts with a different subpopulation.

Two regions were identified in the TSST-1 molecule that affect MHC and/or TCR binding. Those mutations are unambiguously clustered within two sites; the TCR binding site at the C-terminal domain (residues 1-15 and 79-194) and the class II MHC binding site at the N-terminal domain (residues 18-89). The TCR binding site was found to consist of the central long α helix

(see mutations at residues 128-140) and also the N-terminal short α-helix along with its subsequent loop (mutations at Y13L/S15W and G16V). In addition, there is some contribution of residues in the loop between the β7 and β8 sheets (mutations at residues 113-116). Interestingly, the TCR binding site identified in this analysis precisely overlaps with the region comprising the major groove in the TSST-1 molecule (Prasad et al. (1993) Biochemistry 32:13761, hereby incorporated by reference; Acharya et al. (1994) Nature 367:94, hereby incorporated by reference). Amino acid residues at positions 13, 15, 16, 135, 137 and 139 appear to be particularly important for TCR binding, because mutations in these positions almost completely abolish the stimulatory activity of TSST-1 but do not decrease binding to HLA-DR (Figure 4).

The MHC binding site localizes to the region in the vicinity of the N-terminal portion of the second β-strand, because the mutations G31S/S32P greatly impair the binding to HLA-DR (Figures 5A and 5B). These residues are located in part of a large, solvent-exposed hydrophobic area formed by the concave face of β-barrel strands 1, 2 and 3 in the N-terminal domain (see Figure 6). It is noteworthy to point out that the β-barrel motif in the N-terminal domain of TSST-1 is similar to that in the corresponding domain of SEB. In SEB, non-polar residues F44, L45 and F47 form a ridge which protrudes from the loop between β-strands 1 and 2 in the N-terminal domain, and this ridge interacts with the hydrophobic depression in the HLA-DR molecule. SEB mutants F44 and L45 disrupt binding to HLA-DR. Similarly, analysis suggests this hydrophobic region of TSST-1 constitutes the class II MHC binding site. Mutations of S23 on β-strand 1) and I45 (on β-strand 3)

might distort the hydrophobic concave surface, because those residues are buried within the protein interior.

The following examples serve to explain and illustrate the present invention. Said examples are not to be construed as limiting of the invention in any way. Various modifications are possible within the scope of the invention.

Example 1. Construction and Expression of Recombinant TSST-1.

Polymerase Chain Reaction (PCR). PCRs (Saiki et al. (1988) Science 239:487) were performed using AmpliTaq recombinant Taq polymerase and the DNA Thermal Cycler from Perkin Elmer Cetus (Norwalk, CT). 15-17 cycles were performed with 1-min denaturing (94°C) and 2-min annealing (54°C) steps, and a 3-min extension (72°C) step. Template concentrations were 0.5-5 nM and oligonucleotides primer concentrations were 1 μM. The concentration of the dNTPs was 200 μM.

TSST-1 Construct. The gene for superantigen TSST-1 was overexpressed in E. coli as follows. A plasmid containing the mature TSST-1 without the signal peptide (Blomster-Hautamaa et al. (1986) supra) was kindly obtained from Dr. Brian L. Kotzin at The National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado.

The DNA sequence of the cloned mature TSST-1 gene is shown in Figure 1 (SEQ ID NO:1). The gene contains a KpnI site at the 5' portion and a XbaI site at the 3' portion. This gene also contains an ATG codon (encodes methionine) as the translation initiation codon in E. coli.

The plasmid was digested with KpnI and XbaI and ligated into KpnI/XbaI-digested pUC18. E. coli DH5α was transformed with the ligated

DNA, a single transformant picked, and the insert pUC18:TSST-1 was sequenced to check that it had no mutations.

Upon induction the pUC18:TSST-1 construct led to over production of mostly cytoplasmic TSST-1 (≈ 10 µg/ml of broth).

Example 2. Generation of TSST-1 Mutants.

TSST-1 mutants were generated as follows.

A PCR method was used for introducing random mutations in approximately 42-51 base-defined regions (SEQ ID NOS:4-16) of the TSST-1 gene (see Figure 1 and Table 2). The oligonucleotide primers positioned as shown in Figure 1, were synthesized with each position containing 1% each of the three incorrect bases.

These mutant oligonucleotides served as primers in a PCR reaction with a primer designated B bottom primer (see Figure 1) as the other primer, and the TSST-1 gene as the template. Each molecule of synthesized TSST-1 fragment (designated megaprimer, see Figure 2) was predicted to have 1-3 random base mutations in the region corresponding to mutant primer. Mutant fragments were incorporated into the TSST-1 gene with another primer (an A top primer (SEQ ID NO:2) and the XbaI/HindIII digested pUC18:TSST-1 plasmid as the template in the second PCR reaction to resynthesize a full length TSST-1 gene (see Figure 2)(Ho et al. (1989) Gene 77:51; Pullen et al. (1990) Cell 61:1365).

DNA Sequencing. Plasmid inserts were sequenced directly by the dideoxynucleotide method of Sanger et al. (1977) Proc. Natl. Acad. Sci. USA 74:5463, using Sequenase (U.S. Biochemical Corp., Cleveland, OH) and a modification for double-stranded supercoiled plasmid templates (Weickert and

Chambliss (1989) in Editorial Comments, U. S. Biochemical Corp., Cleveland, OH, pg. 5-6).

Example 3. Screening of mutant rTSST-1 bacterial transformants.

Aliquots withdrawn from lysates prepared from 0.2 ml bacterial cultures were tested for protein content and the ability to stimulate T cells. The screening procedure facilitated the determination of a) whether a properly-folded mutant protein molecule had been produced along with its concentration in solution, and b) in an initial screen, whether the mutant molecule stimulated murine TCR V_β15-bearing T cells after presentation by HLA-DR expressing antigen presenting cells (Table 3). Further, the screening procedure confirmed the initial screen in a secondary assay using human peripheral blood leukocytes (hPBL, i.e., a test of mutant molecule activity against all human TCR V_β families).

An enzyme linked immuno-sorbent assay (ELISA) was devised which could yield relative concentrations of folded protein from transformed bacterial cultures (i.e., wild-type rTSST-1 was used as a control in this particular assay)(Table 4). In preliminary experiments, monoclonal antibodies (mAb) were prepared from mice which had been immunized to TSST-1. A panel of eight mAbs were prepared, two of which were used in the ELISA assay for detecting and quantitating mutant rTSST-1. mAbs 327H1.1 (an IgG_{2b}) and 477L1.2 (an IgG_1) were selected as a result of functional assays which showed that 327H1.1 could inhibit TSST-1 binding to HLA-DR molecules, and that 477L1.2 could block T cell recognition of HLA-DR-bound TSST-1. Neither mAb had the complementary activity. It was therefore deduced that 327H1.1 must recognize an epitope on TSST-1 at or

near the HLA-DR association site and 477L1.2 must recognize at or near the TCR V_β interaction site. Since an rTSST-1 molecule having a mutation in one of those regions might be altered such that the mAb binding epitope is lost, the mAbs were used in combination to screen mutant rTSST-1 bacterial lysates.

The ELISA for detecting and quantitating mutant rTSST-1 molecules was carried out by coating microwells of ELISA-grade polystyrene 96-well plates with 2 µg/ml mAb (1 µg/ml each 327H1.1 and 477L1.2) in 0.2 ml coating buffer overnight at 4°C. Unabsorbed mAb was then removed by aspiration, wells were blocked with buffer containing bovine serum albumin (BSA) for 1 hour at 4°C, and then washed three times with buffer solution. Aliquots of bacterial lysate containing mutant rTSST-1, or wild type rTSST-1 for a positive control and lacZ lysate for a negative control, were added to the wells and incubated for 1.5 hours at 37°C. The wells were then washed three times and a polyclonal anti-TSST-1 rabbit IgG:horseradish peroxidase developing antibody added and incubated similarly for 1.5 hours at 37°C. After again washing the wells three times, ortho-phenylenediamine was added for 15-30 minutes at room temperature and color reaction detected on an ELISA plate reader at 490 nm. In this sandwich assay, the developing reagent (the IgG:horseradish peroxidase antibody) will only be present if a rTSST-1 molecule has been bound by the mAb adsorbed to the micro-plate wells. The ability of a mutant rTSST-1 molecule to be bound by mAb which were raised against wild type TSST-1 infers that the mutant has retained some amount of native structural conformation, even if only as much as is necessary to be recognized by the mAb chosen for the assay. Secondly, the amount of color reaction generated in the assay is directly relative to the amount of protein

present, and from which the concentration per ml of mutant rTSST-1 can be calculated.

Mutant rTSST-1 lysates which showed the presence of protein similar to wild type were screened for the ability to stimulate a murine TCR V_β15-positive T hybridoma cell line, KOX15-4.95 (originally prepared in the laboratory of Dr's J. Kappler and P. Marrack at the Howard Hughes Medical Institute, Denver, Colorado) to produce Interleukin-2, IL-2, a T cell growth factor. Dilutions of lysate (corresponding to 3 µl, 0.3 µl and 0.03 µl of the bacterial lysate containing mutant rTSST-1) were added to fresh, sterile 96-well plates containing 1 x 10^5 RAJI cells (an HLA-DR-positive presenting cell) and 1 x 10^5 KOX15-4.95 T hybridoma cells. The cultures were incubated overnight at 37°C whereupon 80 µl of each micro-cultures' supernatant was withdrawn and transferred to a well of a fresh, sterile micro-plate. The IL-2 indicator cell line HT-2 was added to each supernatant (5000 cells/well), incubated overnight at 37°C, and scored the next day for viability. Where no IL-2 was produced, as a result of the inability of a mutant rTSST-1 molecule to stimulate the KOX15-4.95 hybridoma, the HT-2 cells were dead. Alternatively, HT-2 viability indicated that the mutant rTSST-1 isolate was not functionally different from the wild type molecule.

Mutant rTSST-1 lysates which were unable to stimulate the murine KOX15-4.95 hybridoma (i.e., murine V_β15-negative mutants) were rescreened for activity on human normal donor PBL. Dilutions of bacterial lysates, identical to that described above, were cultured with 1 x 10^5 PBL in 0.2 ml micro-cultures for 3 days at 37°C. PBL were solicited from a human volunteer predetermined to have 13% T cells bearing TCR V_β2, the TCR family relevant to TSST-1 binding. Cultures were pulsed overnight with 1

μCi ^{3}H-Tdr, harvested, and incorporated radioactive thymidine quantitated on a scintillation counter. The amount of radioactive label incorporated into cellular DNA is directly related to the amount of cellular proliferation which has transpired during the pulse period. No or little proliferation above background (i.e., PBL cultured without TSST-1) would indicate a mutant rTSST-1 molecule which had lost human T cell stimulatory activity.

rTSST-1 mutants which were negative for activity on both murine TCR V_β15 and human PBL were re-cultured from cloned bacterial transformants and purified by HPLC to obtain milligram amounts of purified protein (see Example 4). These HPLC-purified mutant proteins were then tested for the ability to stimulate human PBL proliferation *in vitro*, as described above, except that in the present assay, wild type and mutants were added to micro-wells in concentrations from 1.0 fg/ml to 100 μg/ml. The data obtained (Figure 4) illustrates the difference in ability of high concentrations of mutant protein to stimulate human PBL compared to the wild type rTSST-1.

Example 4. Preparation of Recombinant TSST-1.

For initial screening, individual colonies of transformants picked from agar plates were transferred to wells of 96-well microtiter plates containing 100 μl of 2XYT and carbenicillin. A replicate plate was prepared except that the media contained 1 mM IPTG as well. Both were incubated overnight at 37°C. 50 μl of glycerol was added to each well of the first plate, which was mixed and then stored at -70°C. To prepare TSST-1-containing lysates, each well of the second plate received 50 μl of HNM buffer (10 mM Hepes, pH 7.0, 30 mM NaCl, 5 mM $MgCl_2$) containing 3 mg/ml lysozyme and 300 μg/ml DNAse I. The plate was incubated at 37°C for 15 min, frozen and thawed

three times, and centrifuged t pellet debris. The supernatants were transferred to a new plate containing an equal volume of HNM buffer and tested for the presence of TSST-1 both by ELISA and T cell hybridoma stimulation. This method produced preparations containing 1 to 10 μg/ml of TSST-1.

To produce purified mutant TSST-1, transformants were recovered from the 96-well plate stored at -70°C. Bacteria from overnight cultures (typically 200 ml) induced by the addition of 1 mM IPTG were collected by centrifugation and resuspended in a 10 ml of TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA). The cells were then disrupted by an ultrasonic oscillation for 3 minutes on ice and incubated with 200 μg of DNAse I for 30 min at 37°C. The suspension was centrifuged at 15,000 g for 20 min to remove bacterial debris, and the supernatant was harvested and filtered (0.45 nm). The filtrate (~12 ml) was concentrated to ~ 1 ml with an Amicon Centriprep (10,000 MW cut-off) by the centrifugation at 5,000 g for 1 hr. The concentrate was dialyzed overnight at 4°C against 1 liter of 10 mM sodium phosphate buffer, pH 6.1. The dialyzed cell extract was then applied to a cation exchange high-performance liquid chromatography (HPLC) column (SP-5-PW, 7.5 mm x 75 mm; BioRad), equilibrated with 10 mM sodium phosphate buffer, pH 6.1 and eluted with a linear gradient of 0 to 500 mM NaCl in 10 mM sodium phosphate buffer, pH 6.1. The TSST-1 was eluted at about 120 mM NaCl. This method yielded 5-10 mg of toxin per liter of bacterial culture. TSST-1 and its mutant produced in this manner was >95% pure as judged by SDS-PAGE.

Example 5. Binding of Mutant TSST-1 to HLA-DR.

Binding to class II MHC is frequently required for T cell stimulation and subsequent toxin recognition by T cells. Thus, the mutations of the instant invention could have affected either the ability of the toxin to bind to DR molecules or the recognition of this complex by the TCR. To help distinguish these two possibilities, T cell stimulatory-negative rTSST-1 mutants were tested for the ability to bind to HLA-DR. In this assay, RAJI-DR cells, a human B cell tumor line which expresses a high and homogenous level of HLA-DR, were incubated with titrated amounts of toxin in buffer containing azide on ice for 2 hours. After washing to remove unbound toxin, biotinylated anti-TSST-1 mAb 93E1.2 was added for 30 minutes, on ice. After a second wash to remove unbound mAb, phycoerythrin:avidin was added for 15-30 minutes, on ice, to allow mAb detection. Samples were evaluated for specific immunofluorescence, indicating TSST-1 binding to HLA-DR as compared to nil TSST background controls, on a flow cytometer.

Only one mutant rTSST-1 isolate failed to bind to HLA-DR on RAJI cells (the mutant G31S/S32P, see Figure 5A). To confirm the lack of binding as detected by the anti-TSST-1 mAb 93E1.2, the G31S/S32P protein was directly biotinylated and tested versus biotinylated wild-type TSST-1 for activity. As shown in Figure 5B, the G31S/S32P mutant protein does bind HLA-DR, but only at 4-5 log higher protein concentrations than can be demonstrated using the wild type toxin. In addition, G31S/S32P binding does not approach saturation of the RAJI cells. That is, it labeled less than 40% cells at 10 µg/ml compared to the wild type toxin which labeled >90% cells at protein concentrations of 0.01 µg/ml. The binding of TSST-1 is severely affected, but not completely abrogated, by the mutations at G31S/S32P (Gly 31 → Ser/Ser 32 → Pro).

Structural Studies of TSST-1.

The three-dimensional crystal structure of TSST-1 has been recently reported (Prasad et al. (1993) supra; Acharya et al. (1994) supra). A schematic drawing of TSST-1 is shown in Figure 6. The TSST-1 molecule contains two domains. The first is composed of residues 1-15 and 79-194 (the N-terminal domain), and the second of residues 18-89(the C-terminal domain). As discussed above, two regions have been identified in TSST-1 that affect MHC class II binding and/or T cell activation. In each of the regions the specific amino acids that are responsible were determined. Some of the identified residues affect MHC class II binding, whereas others affect T cell activation. As superantigen-MHC class II binding is required for efficient T cell activation in culture, residues affecting MHC class II binding will also influence T cell activation. Thus, no T cell binding information can be inferred from them. Those residues do provide information about MHC class II binding sites on the superantigen. On the other hand, those residues that influence T cell activation but not MHC class II binding are likely to be in the TCR binding site on TSST-1.

Additionally, Figure 6 shows the locations of mutations that abolished T cell stimulation activity of TSST-1. As shown in the figure, these mutations are clustered in the two distinct sites; TCR binding site in the C-terminal domain and MHC binding site in the N-terminal domain. The TCR binding site consists of the C-terminal portion of helix A and its subsequent loop (mutations at residues 13-16), helix B (mutations at residues 131-140) and the loop preceding strand 9 (mutations at residues 113-116). Interestingly, the TCR binding site identified in this study precisely overlaps with the region

comprising the major groove in the TSST-1 structure (Prasad et al. (1993) supra). Namely the groove, which is profound, is formed by the walls rising from helix B, by helix A with its subsequent loop before strand 1 and by strand 9 with is preceding loop. Thus, the entire groove in the middle of the TSST-1 molecule appears to provide the TCR binding site. Among these residues, the residues at positions 13, 15, 16, 132, 135, 136 and 139 are particularly important for the TCR binding, as the mutations in these positions (mutants G16V, Y13L/S15W, H135R, E132A/Q136L, Q139P1, AND Q139L/Y144F) almost completely abolished the superantigenicity of TSST-1 (see Figure 4).

The MHC binding site appears to be located in the proximity of the N-terminal portion of strand 2, since the mutant G31S/S32P failed to bind to DR1 bearing RAJI cells (Figures 5A and 5B). This region is in a part of the large, solvent-exposed hydrophobic area (300 $Å^2$) formed by the concave face of strands 1, 2 and 3 (Prasad et al. (1993) supra; Acharya et al. (1994) supra). The residues are involved are Ser 29, Leu 30, Ser 32, Leu 44, Ile 46, Pro 48, and Pro 50. It is noteworthy to point out that the beta-barrel motif of the N-terminal domain of TSST-1 is similar to the corresponding domain of SEB, and a ridge of non-polar residues (Phe 44, Leu 45 and Phe 47) protruding from the loop between strands 1 and 2 of the SEB molecule interacts with the hydrophobic region of MHC class II (HLA-DR1) molecule (Jardetzky et al. (1994) supra). Mutations of residues Phe 44 and Leu 45 in the SEB molecule disrupt binding to HLA-DR1 (Kappler et al. (1992) J. Exp. Med. 175:387). Thus, it is most likely that the hydrophobic region formed by the carboxyl end of strands 1, 3 and 5 and the amino ends of strand 2 of the TSST-1 molecule is the MHC binding site. It is not clear, however, why residues at 23 (strand 1)

and 45 (strand 3), which are both buried within the protein interior, have diminished T cell activation capability. This is not to be limited by speculation. These mutations might disrupt local structure in the concave surface of strands 1, 2 and 3, so that the mutant TSST-1 cannot interact with MHC class II with high affinity.

Example 6. The protective effects of TSST-1 mutants in animals.

Protective immunity in non-human primates elicited by a superantigen vaccine has been previously demonstrated (Tseng, et al. (1993) supra). In addition, superantigen mutant molecules devoid of T-stimulatory activity have been shown to be unable to elicit the physiological symptoms of disease (Harris, et al. (1993) Inf. & Immun. 61:3175). These two criteria; a) to confer protective immunity against the wild type toxin, and b) to lack disease-causative capability, may be demonstrated for a rTSST-1 mutant molecule, the vaccine.

All experiments are based on a toxemia model (as described by Murray, et al. (1994) J. Immunol. 152:87), where 200 μg TSST-1 protein/0.2 ml PBS in miniosmotic pumps are implanted sub-cutaneously in rabbits. Wild type TSST-1 results in 100% animal death in 7 days. Subject animals can also be monitored for symptoms of TSS, including diarrhea, conjunctival and ear redness, rectal temperature, and serum glutamic pyruvic transaminase and blood urea nitrogen levels for indication of hepatic and renal failure, respectively. The toxemia model experiment is designed to compare the TSST-1 "vaccine" against the wild type toxin. This experiment is then repeated to include a toxicity limit test. That is, determine the maximum

TSST-1 vaccine dosage. Finally, the vaccinated rabbits are challenged with wild-type TSST-1 to determine the protection conferred by the vaccine.

TABLE I. KNOWN SUPERANTIGEN SEQUENCES AND STRUCTURES

Staphylococcus	Staphylococcal enterotoxin A	Huang et al. (1987) J. Biol. Chem. 262:7006 Betley et al. (1988) J. Bacteriol. 170:34
	Staphylococal enterotoxin B	Jones & Khan (1986) J. Bacteriol. 166:29 Huang & Bergdoll (1970) J. Biol. Chem. 245:3518 Ranelli et al. (1985) Proc. Natl. Acad. Sci. 82:5850
	Staphylococal enterotoxin C1 and C3	Schmidt & Spero (1983) J. Biol. Chem. 258:6300 Bohach & Schlievert (1987) Mol. Gen. Genet. 209:15 Couch & Betley (1989) J. Bacteriol. 171:4507
	Staphylococal enterotoxin D	Bayles & Iandolo (1989) J. Bacteriol. 171:4799
	Staphylococcal enterotoxin E	Couch et al. (1989) J. Bacteriol. 170:2954
Toxic Shock Toxin		Schlievert et al. (1981) J. Infect. Dis. 143:509 Blomster-Hautamaa et al. (1986) J. Biol. Chem. 261:15783 Bergdoll et al. (1981) Lancet 1:1017
Exfoliating Toxins		Lee et al. (1987) J. Bacteriol. 169:3904
Streptococcus	Streptococcal Pyrogenic Toxin C	Goshorn & Schlievert (1988) Infect. Immun. 56:2518 Tomai et al. (1990) J. Exp. Med. 172:359
Mouse Mammary Tumor Virus		Fasel et al. (1982) EMBO J. 1:30 Donehower et al. (1981) J. Virol. 37:226 Donehower et al. (1983) J. Virol. 45:941 Raceviskis & Prakash (1984) J. Virol. 51:604 Choi et al. (1991) Nature 350:203 Acha-Orbea et al. (1991) Nature 350:207 Pullen et al. (1992) J. Exp. Med. 175:41 Moore et al. (1987) J. of Virology 61:480

Table 2. DNA sequences of primers used for mutagenesis of TSST-1

No.	Residues subject to mutagenesis	DNA sequence of mutagenic primer	
1	1 - 17	5'-TCT ACA AAC GAT AAT ATA AAG GAT TTG CTA GAC TGG TAT AGT AGT GGG TCT-3'	(SEQ ID NO:4)
2	16 - 32	5'-GGG TCT GAC ACT TTT ACA AAT AGT GAA GTT TTA GAT AAT TCC TTA GGA TCT-3'	(SEQ ID NO:5)
3	31 - 47	5'-GGA TCT ATG CGT ATA AAA AAC ACA GAT GGC AGC ATC AGC CTT ATA ATT TTT-3'	(SEQ ID NO:6)
4	46 - 62	5'-ATT TTT CCG AGT CCT TAT TAT AGC CCT GCT TTT ACA AAA GGG GAA AAA GTT-3'	(SEQ ID NO:7)
5	61 - 77	5'-AAA GTT GAC TTA AAC ACA AAA AGA ACT AAA AAA AGC CAA CAT ACT AGC GAA-3'	(SEQ ID NO:8)
6	76 - 92	5'-AGC GAA GGA ACT TAT ATC CAT TTC CAA ATA AGT GGC GTT ACA AAT ACT GAA-3'	(SEQ ID NO:9)
7	91 - 107	5'-ACT GAA AAA TTA CCT ACT CCA ATA GAA CTA CCT TTA AAA GTT AAG GTT CAT-3'	(SEQ ID NO:10)
8	106 - 122	5'-GTT CAT GGT AAA GAT AGC CCC TTA AAG TAT TGG CCA AAG TTC GAT AAA AAA-3'	(SEQ ID NO:11)
9	121 - 137	5'-AAA AAA CAA TTA GCT ATA TCA ACT TTA GAC TTT GAA ATT CGT CAT CAG CTA-3'	(SEQ ID NO:12)
10	136 - 152	5'-CAG CTA ACT CAA ATA CAT GGA TTA TAT CGT TCA AGC GAT AAA ACG GGT GGT-3'	(SEQ ID NO:13)
11	151 - 167	5'-GGT GGT TAT TGG AAA ATA ACA ATG AAT GAC GGA TCC ACA TAT CAA AGT GAT-3'	(SEQ ID NO:14)
12	166 - 182	5'-AGT GAT TTA TCT AAA AAG TTT GAA TAC AAT ACT GAA AAA CCA CCT ATA AAT-3	(SEQ ID NO:15)
13	181 - 194	5'-ATA AAT ATT GAT GAA ATA AAA ACT ATA GAA GCA GAA ATT AAT-3'	(SEQ ID NO:16)

Table 3. Mutation frequencies of murine Vβ15-bearing T cell hybridoma stimulation negative TSST-1

Library No.	TSST-1 residues subject to mutation	Murine Vβ15 stimulation (negative/total)	%
1	1 -17	10 / 282	3.5
2	16 - 32	17 / 282	6.0
3	31 - 47	8 / 282	2.8
4	46 - 62	0 / 94	0.0
5	61 - 77	0 / 94	0.0
6	76 - 92	0 / 60	0.0
7	91 - 107	6 / 282	2.1
8	106 - 122	7 / 282	2.5
9	121 - 137	6 / 96	6.3
10	136 - 152	33 / 282	11.7
11	151 - 167	0 / 94	0.0
12	166 - 182	1 / 107	0.9
13	181 - 194	0 / 35	0.0

Table 4. Summary of TSST-1 point mutants

Murine Vβ15 non-stimulatory mutants	Response to human PBL	Binding to HLA-DR1
L10Q/D11V	++++	
Y13L/S15W	-	++++
S14I	++	
S15G	++++	
G16V	-	++++
T21R	++++	
S23R	++++	
S23R/E24V	++	
G31S/S32P	-	+
S41T	++++	
I45V	++++	
P97L/L102V	++++	
L102S	++++	
L113F/K114T	++++	
W116R, S	+++	
F131IV	++++	
E132A/Q136L	++	++++
H135R	-	++++
Q136R	+++	++++
Q139L, P	++, - (Q139P)	++++ (Q139P)
Q139E/L143S	-	++++
Q139E/S147R	+	
Q139L/Y144F	-	++++
Q139L/Y144N	+	
I140V	++++	
H141L/S147G	++++	
R145H, P, S	++++	

All mutant proteins listed here failed to stimulate murine Vβ15 T hybridoma, yet produced wild-type levels of rTSST-1 as determined by ELISA. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. Data is presented as, "++++" = 75-100%; "+++" = 50-75%; "++" = 25-50%; "+" = 10-25%; and "-" = <10% of the response to wild-type toxin.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: PHILLIPA MARRACK
JOHN KAPPLER
RICHARD SHIMONKEVITZ
MASAZUMI MATSUMURA

(ii) TITLE OF INVENTION: PROTECTIVE EFFECTS OF MUTATED SUPERANTIGENS

(iii) NUMBER OF SEQUENCES: 16

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 8400 E. Prentice Ave., Suite 200
(C) CITY: Englewood
(D) STATE: Colorado
(E) COUNTRY: USA
(F) ZIP: 80111

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3.5 inch
(B) COMPUTER: IBM pc compatible
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WordPerfect 6.0 for windows

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/338,373
(B) FILING DATE: 14-November-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Barry J. Swanson
(B) REGISTRATION NUMBER: 33,215
(C) REFERENCE/DOCKET NUMBER: SUP020/PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (303) 793-3333
(B) TELEFAX: (303) 793-3433

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 657 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAATTCGAGC TCGGTACCCC GAAGGAGGAA AAAAAA ATG TCT ACA AAC          48
                                         Met Ser Thr Asn
                                          1
GAT AAT ATA AAG GAT TTG CTA GAC TGG TAT AGT AGT GGG TCT          90
Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser Gly Ser
    5                  10                  15
GAC ACT TTT ACA AAT AGT GAA GTT TTA GAT AAT TCC TTA GGA         132
Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu Gly
        20                  25                  30
TCT ATG CGT ATA AAA AAC ACA GAT GGC AGC ATC AGC CTT ATA         174
Ser Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile
            35                  40                  45
ATT TTT CCG AGT CCT TAT TAT AGC CCT GCT TTT ACA AAA GGG         216
Ile Phe Pro Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly
                50                  55
GAA AAA GTT GAC TTA AAC ACA AAA AGA ACT AAA AAA AGC CAA         258
Glu Lys Val Asp Leu Asn Thr Lys Arg Thr Lys Lys Ser Gln
60                  65                  70
CAT ACT AGC GAA GGA ACT TAT ATC CAT TTC CAA ATA AGT GGC         300
His Thr Ser Glu Gly Thr Tyr Ile His Phe Gln Ile Ser Gly
    75                  80                  85
GTT ACA AAT ACT GAA AAA TTA CCT ACT CCA ATA GAA CTA CCT         342
Val Thr Asn Thr Glu Lys Leu Pro Thr Pro Ile Glu Leu Pro
        90                  95                  100
TTA AAA GTT AAG GTT CAT GGT AAA GAT AGC CCC TTA AAG TAT         384
Leu Lys Val Lys Val His Gly Lys Asp Ser Pro Leu Lys Tyr
            105                 110                 115
TGG CCA AAG TTC GAT AAA AAA CAA TTA GCT ATA TCA ACT TTA         426
Trp Pro Lys Phe Asp Lys Lys Gln Leu Ala Ile Ser Thr Leu
                120                 125
GAC TTT GAA ATT CGT CAT CAG CTA ACT CAA ATA CAT GGA TTA         468
Asp Phe Glu Ile Arg His Gln Leu Thr Gln Ile His Gly Leu
130                 135                 140
TAT CGT TCA AGC GAT AAA ACG GGT GGT TAT TGG AAA ATA ACA         510
Tyr Arg Ser Ser Asp Lys Thr Gly Gly Tyr Trp Lys Ile Thr
```

```
    145                 150                 155
ATG AAT GAC GGA TCC ACA TAT CAA AGT GAT TTA TCT AAA AAG        552
Met Asn Asp Gly Ser Thr Tyr Gln Ser Asp Leu Ser Lys Lys
        160                 165                 170
TTT GAA TAC AAT ACT GAA AAA CCA CCT ATA AAT ATT GAT GAA        594
Phe Glu Tyr Asn Thr Glu Lys Pro Pro Ile Asn Ile Asp Glu
            175                 180                 185
ATA AAA ACT ATA GAA GCA GAA ATT AAT TAA TGCTCTAGAG             634
Ile Lys Thr Ile Glu Ala Glu Ile Asn
                190
TCGACCTGCA GGCATGCAAG CTT                                      657
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
GAATTCGAGC TCGGTACCCC GAAGGAGGAA AAAAAA                        36
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
TGCTCTAGAG TCGACCTGCA GGCATGCAAG CTT                           33
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
TCT ACA AAC GAT AAT ATA AAG GAT TTG CTA GAC TGG TAT AGT AGT    45
Ser Thr Asn Asp Asn Ile Lys Asp Leu Leu Asp Trp Tyr Ser Ser
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GGG TCT GAC ACT TTT ACA AAT AGT GAA GTT TTA GAT AAT TCC TTA    45
Gly Ser Asp Thr Phe Thr Asn Ser Glu Val Leu Asp Asn Ser Leu
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GGA TCT ATG CGT ATA AAA AAC ACA GAT GGC AGC ATC AGC CTT ATA    45
Gly Ser Met Arg Ile Lys Asn Thr Asp Gly Ser Ile Ser Leu Ile
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
ATT TTT CCG AGT CCT TAT TAT AGC CCT GCT TTT ACA AAA GGG GAA    45
Ile Phe Pro Ser Pro Tyr Tyr Ser Pro Ala Phe Thr Lys Gly Glu
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AAA GTT GAC TTA AAC ACA AAA AGA ACT AAA AAA AGC CAA CAT ACT 45
Lys Val Asp Leu Asn Thr Lys Arg Thr Lys Lys Ser Gln His Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGC GAA GGA ACT TAT ATC CAT TTC CAA ATA AGT GGC GTT ACA AAT 45
Ser Glu Gly Thr Tyr Ile His Phe Gln Ile Ser Gly Val Thr Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACT GAA AAA TTA CCT ACT CCA ATA GAA CTA CCT TTA AAA GTT AAG 45
Thr Glu Lys Leu Pro Thr Pro Ile Glu Leu Pro Leu Lys Val Lys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTT CAT GGT AAA GAT AGC CCC TTA AAG TAT TGG CCA AAG TTC GAT 45
Val His Gly Lys Asp Ser Pro Leu Lys Tyr Trp Pro Lys Phe Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
AAA AAA CAA TTA GCT ATA TCA ACT TTA GAC TTT GAA ATT CGT CAT    45
Lys Lys Gln Leu Ala Ile Ser Thr Leu Asp Phe Glu Ile Arg His
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
CAG CTA ACT CAA ATA CAT GGA TTA TAT CGT TCA AGC GAT AAA ACG    45
Gln Leu Thr Gln Ile His Gly Leu Tyr Arg Ser Ser Asp Lys Thr
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
GGT GGT TAT TGG AAA ATA ACA ATG AAT GAC GGA TCC ACA TAT CAA    45
Gly Gly Tyr Trp Lys Ile Thr Met Asn Asp Gly Ser Thr Tyr Gln
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
AGT GAT TTA TCT AAA AAG TTT GAA TAC AAT ACT GAA AAA CCA CCT    45
Ser Asp Leu Ser Lys Lys Phe Glu Tyr Asn Thr Glu Lys Pro Pro
```

1 5 10 15

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATA AAT ATT GAT GAA ATA AAA ACT ATA GAA GCA GAA ATT AAT 42
Ile Asn Ile Asp Glu Ile Lys Thr Ile Glu Ala Glu Ile Asn
1 5 10

CLAIMS

We Claim:

1. A method for modifying T cell responsiveness of a subject animal without eliciting a superantigen-mediated response comprising administering to said subject animal a superantigen or superantigen derivative in an amount sufficient to modify the responsiveness of at least one subpopulation of T cells.

2. A molecule for modifying the T cell responsiveness of a subject animal comprising a superantigen or superantigen derivative capable of conferring protection against exposure to a superantigen by eliciting an antibody response to said superantigen in said subject animal.

4. The method of claim 1, wherein said modifying comprises reducing responsiveness of at least one subpopulation of T cells of said subject animal to an antigen.

5. The method of claim 1, wherein said modifying comprises increasing responsiveness of at least one subpopulation of T cells of said subject animal to an antigen.

6. The method of claim 1, wherein said modifying comprises reducing responsiveness of at least one subpopulation of T cells of said subject animal to an antigen without stimulating said T cells in a superantigenic way.

7. The method of claim 1, wherein said superantigen derivative is a modified superantigen fragment.

8. The method of claim 1, wherein said superantigen derivative is a mutated superantigen or mutated superantigen fragment.

9. The method of claim 8, wherein said mutated superantigen or mutated superantigen fragment is the result of a substitution mutation.

10. The method of claim 8, wherein said mutated superantigen is the result of a deletion mutation.

11. The method of claim 7, wherein said superantigen derivative is a molecule containing a portion of an amino acid sequence of a superantigen and a portion of said derivative is not derived from a superantigen.

12. The method of claim 1, wherein said superantigen derivative is a Staphylococcus aureus superantigen derivative.

13. The method of claim 1, wherein said superantigen derivative is a Staphylococcus TSST-1 superantigen or superantigen derivative.

14. A method for conferring protection against Toxic Shock Syndrome Toxin (TSST-1) comprising administering to a subject animal an amount of a TSST-1 derivative protein.

15. The method of claim 1, wherein said subpopulation comprises the requisite specificity as defined by its V_β region.

16. The molecule of claim 2, wherein said molecule is a mutated superantigen derivative.

17. The molecule of claim 16, wherein said molecule is a mutated Staphylococcus superantigen derivative.

18. The molecule of claim 2, wherein said molecule is a modified superantigen derivative.

19. The molecule of claim 18, wherein said molecule is a modified Staphylococcus superantigen derivative.

20. The molecule of claim 17, wherein said Staphylococcus superantigen derivative is a mutated TSST-1 superantigen

21. The molecule of claim 19, wherein said Staphylococcus superantigen derivative is a modified TSST-1 superantigen.

22. The Staphylococcus TSST-1 superantigen derivative of claim 20, wherein said superantigen derivative modifies T cell receptor or class II major histocompatibility complex binding upon administration to a subject animal.

23. The Staphylococcus TSST-1 superantigen derivative of claim 21, wherein said superantigen derivative modifies T cell receptor or class II major histocompatibility complex binding upon administration to a subject animal.

24. The Staphylococcus TSST-1 superantigen derivative of claim 20, wherein said superantigen differs from normal TSST-1 superantigen in at least one amino acid from amino acids 1-15 or 79-194 and at least one amino acid from amino acids 18-89 of normal TSST-1.

25. The Staphylococcus TSST-1 superantigen derivative of claim 21, wherein said superantigen differs from normal TSST-1 superantigen in at least one amino acid from amino acids 1-15 or 79-194 and at least one amino acid from amino acids 18-89 of normal TSST-1.

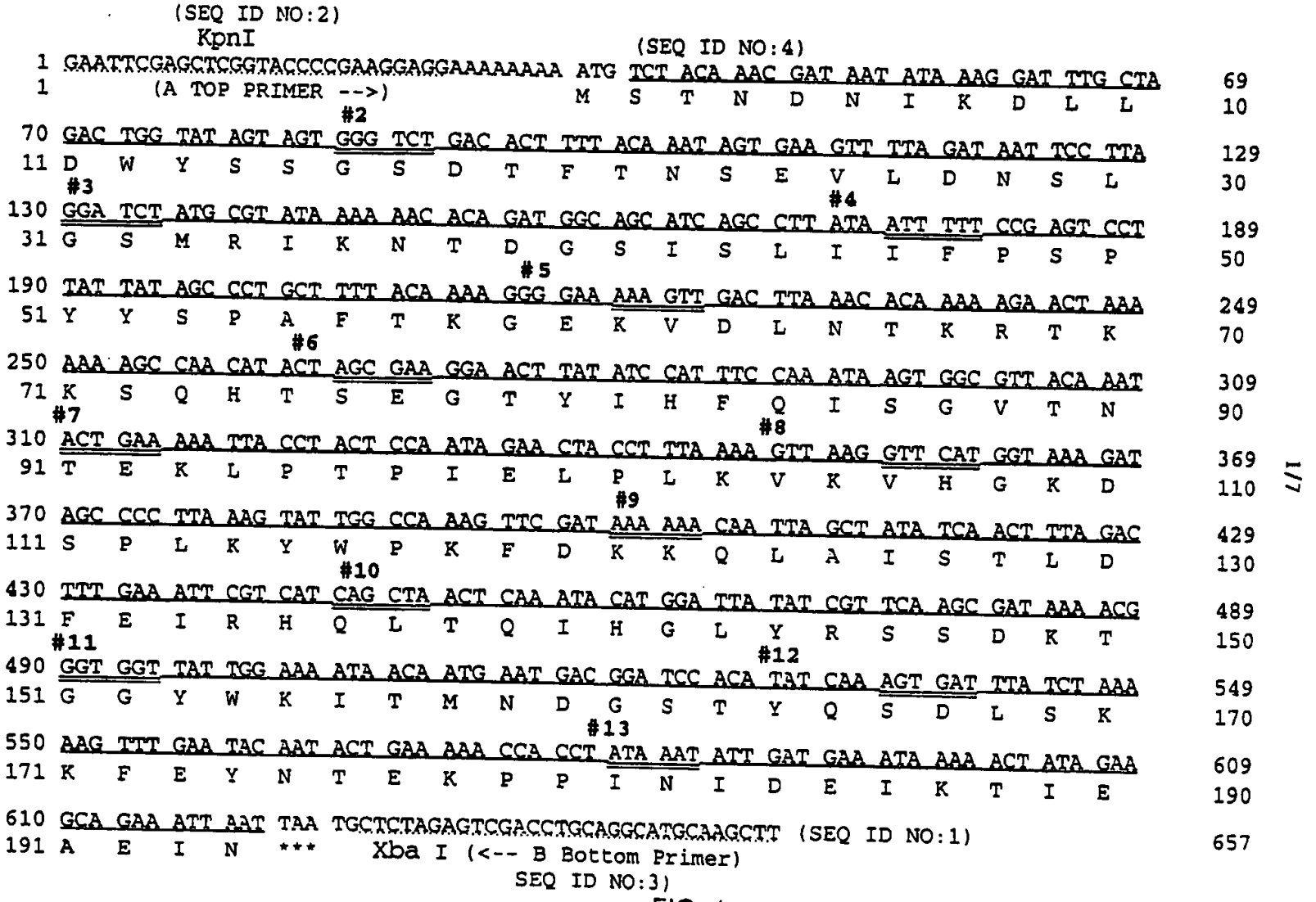

(SEQ ID NO:2)
KpnI
(SEQ ID NO:4)
(A TOP PRIMER -->)
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13
Xba I (<-- B Bottom Primer)
SEQ ID NO:3)
(SEQ ID NO:1)

FIG. 1

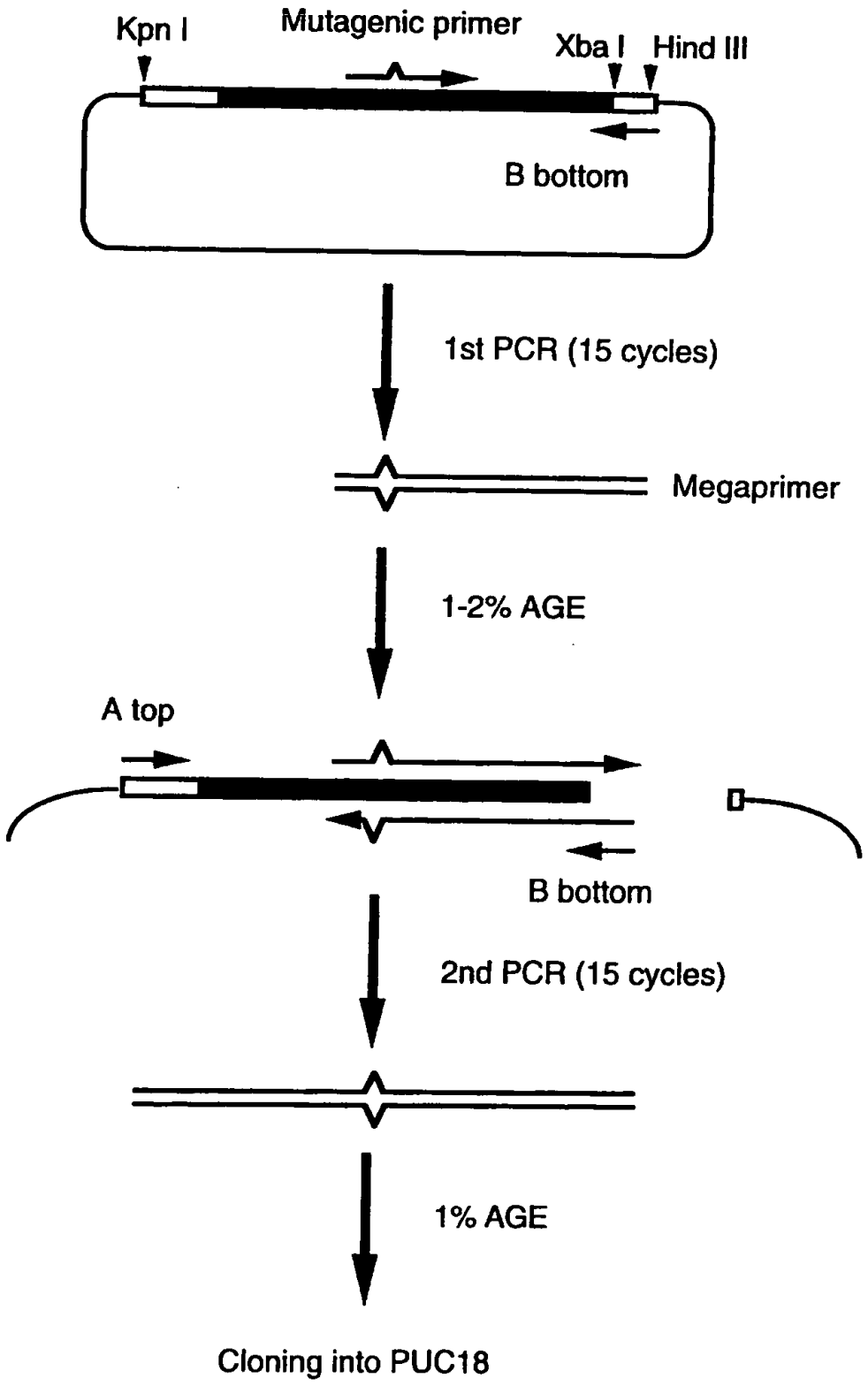

Kpn I
Mutagenic primer
Xba I
Hind III
B bottom
1st PCR (15 cycles)
Megaprimer
1-2% AGE
A top
B bottom
2nd PCR (15 cycles)
1% AGE
Cloning into PUC18

FIG. 2

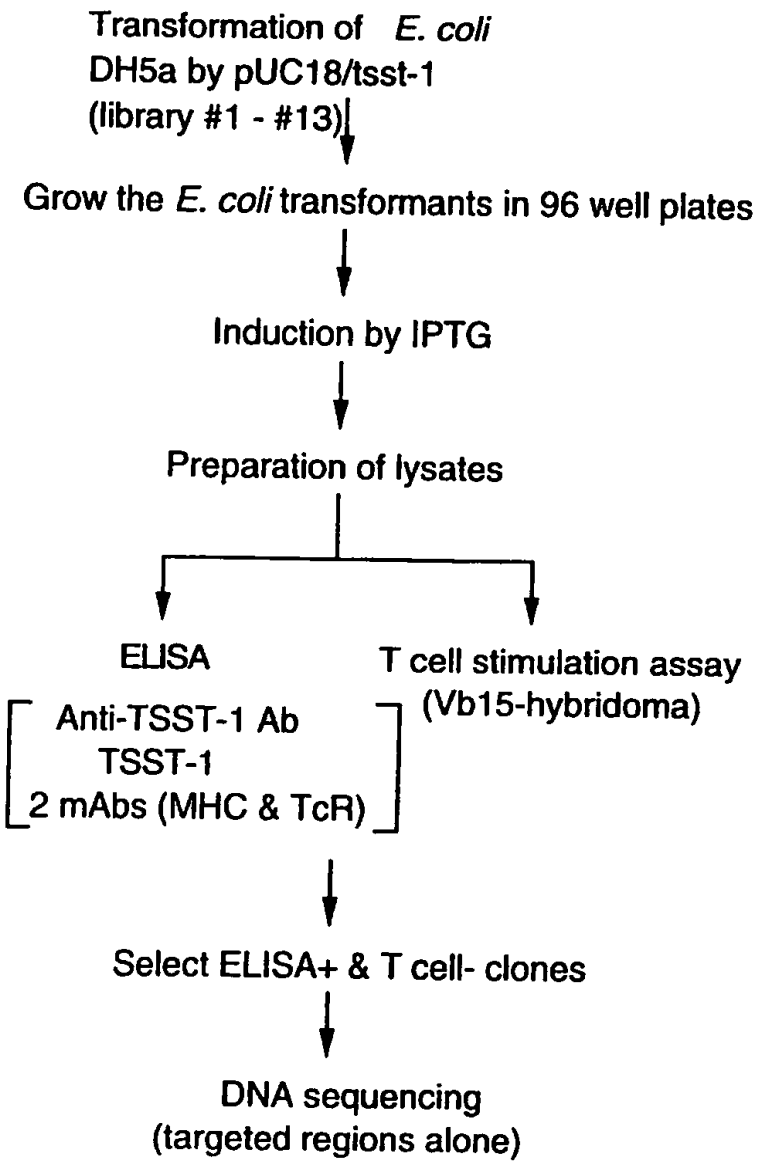

Transformation of E. coli
DH5a by pUC18/tsst-1
(library #1 - #13)
Grow the E. coli transformants in 96 well plates
Induction by IPTG
Preparation of lysates
ELISA
Anti-TSST-1 Ab
TSST-1
2 mAbs (MHC & TcR)
T cell stimulation assay
(Vb15-hybridoma)
Select ELISA+ & T cell- clones
DNA sequencing
(targeted regions alone)

FIG. 3

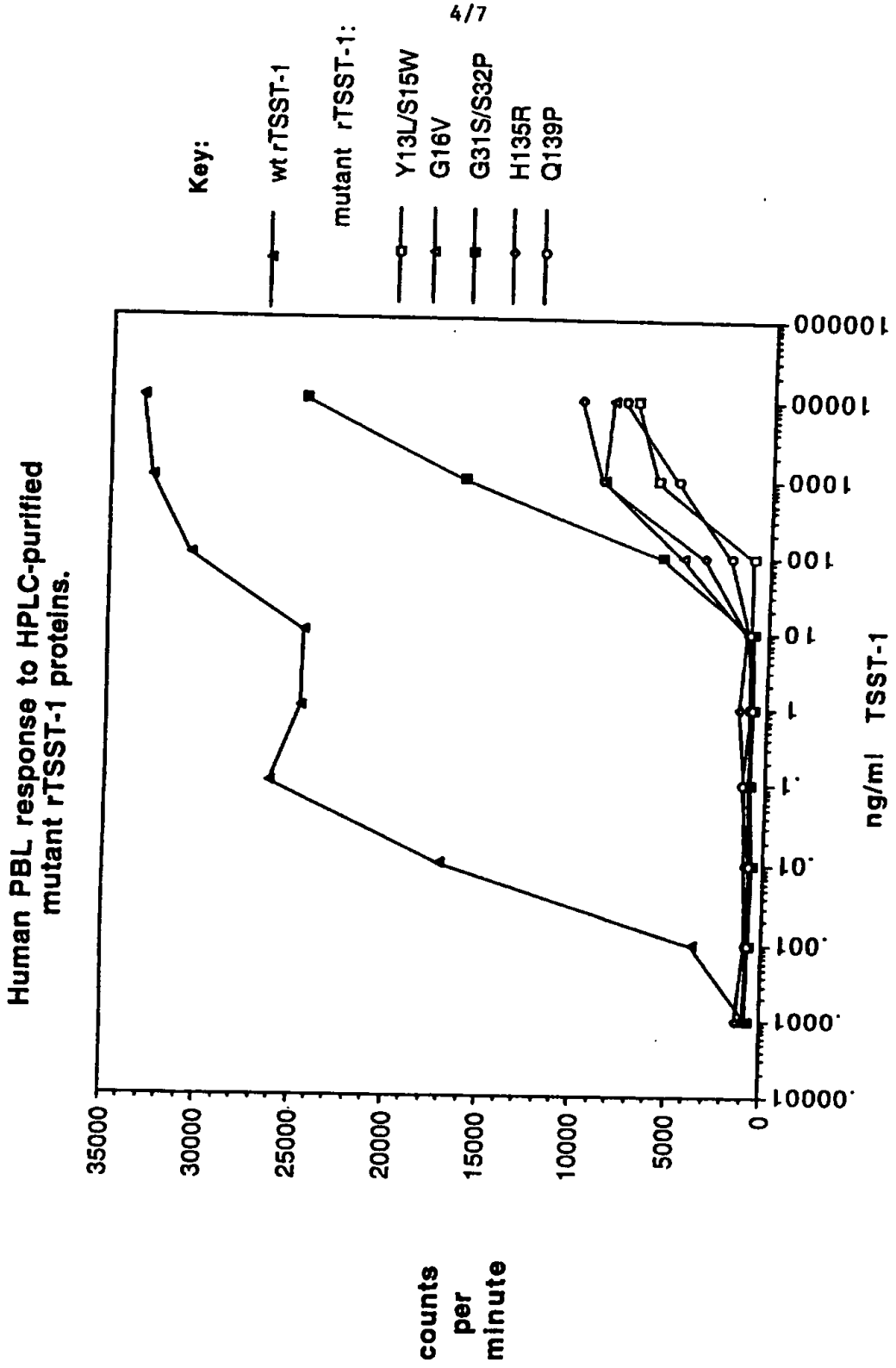

Human PBL response to HPLC-purified mutant rTSST-1 proteins.
Key:
wt rTSST-1
mutant rTSST-1:
Y13L/S15W
G16V
G31S/S32P
H135R
Q139P
counts per minute
35000
30000
25000
20000
15000
10000
5000
0
100000
10000
1000
100
10
1
.1
.01
.001
.0001
.00001
ng/ml TSST-1

FIG. 4

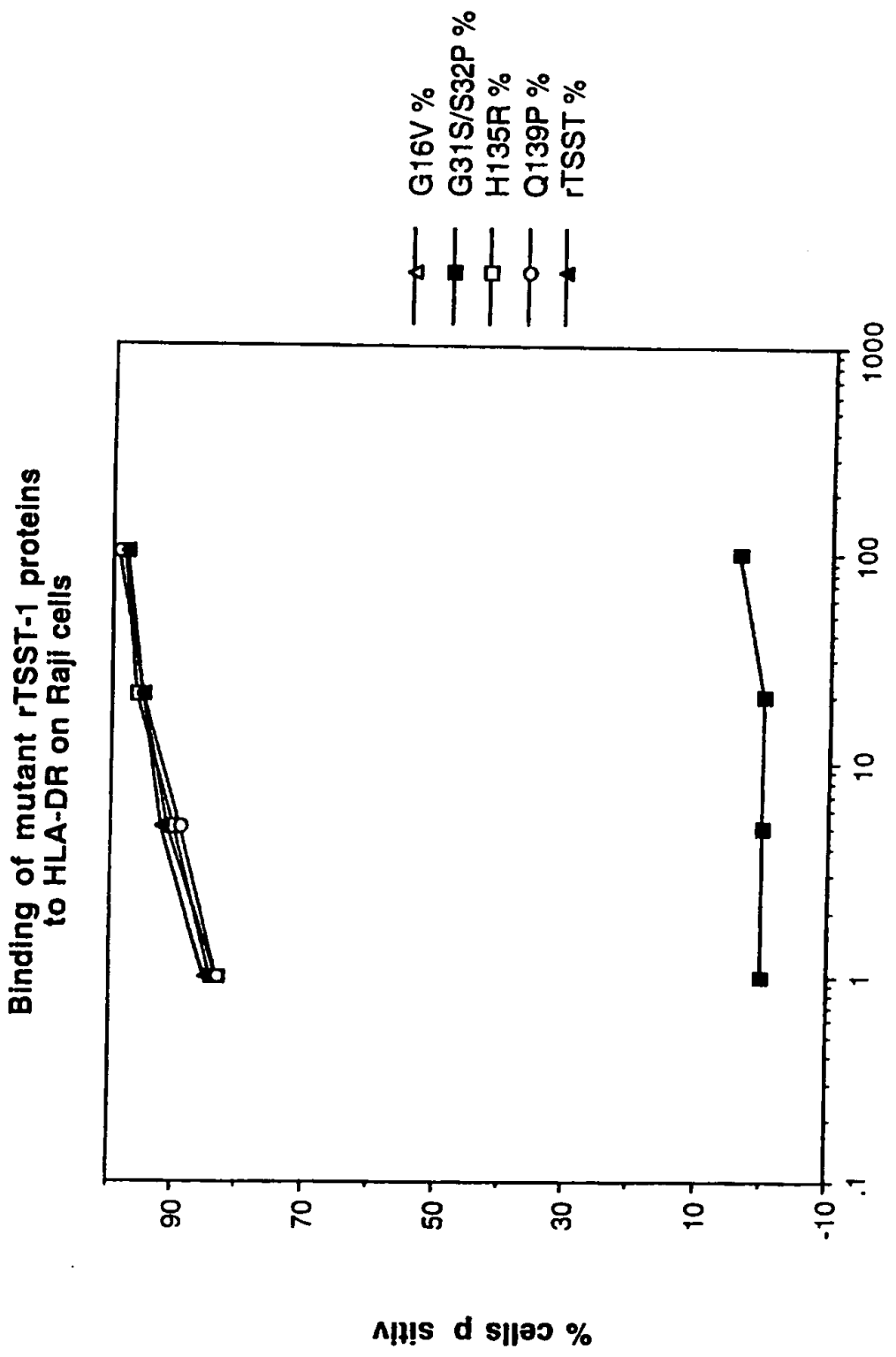

Binding of mutant rTSST-1 proteins to HLA-DR on Raji cells
G16V %
G31S/S32P %
H135R %
Q139P %
rTSST %
% cells positivity
90
70
50
30
10
-10
.1
1
10
100
1000
ug/ml mutant or wild type protein

FIG. 5A

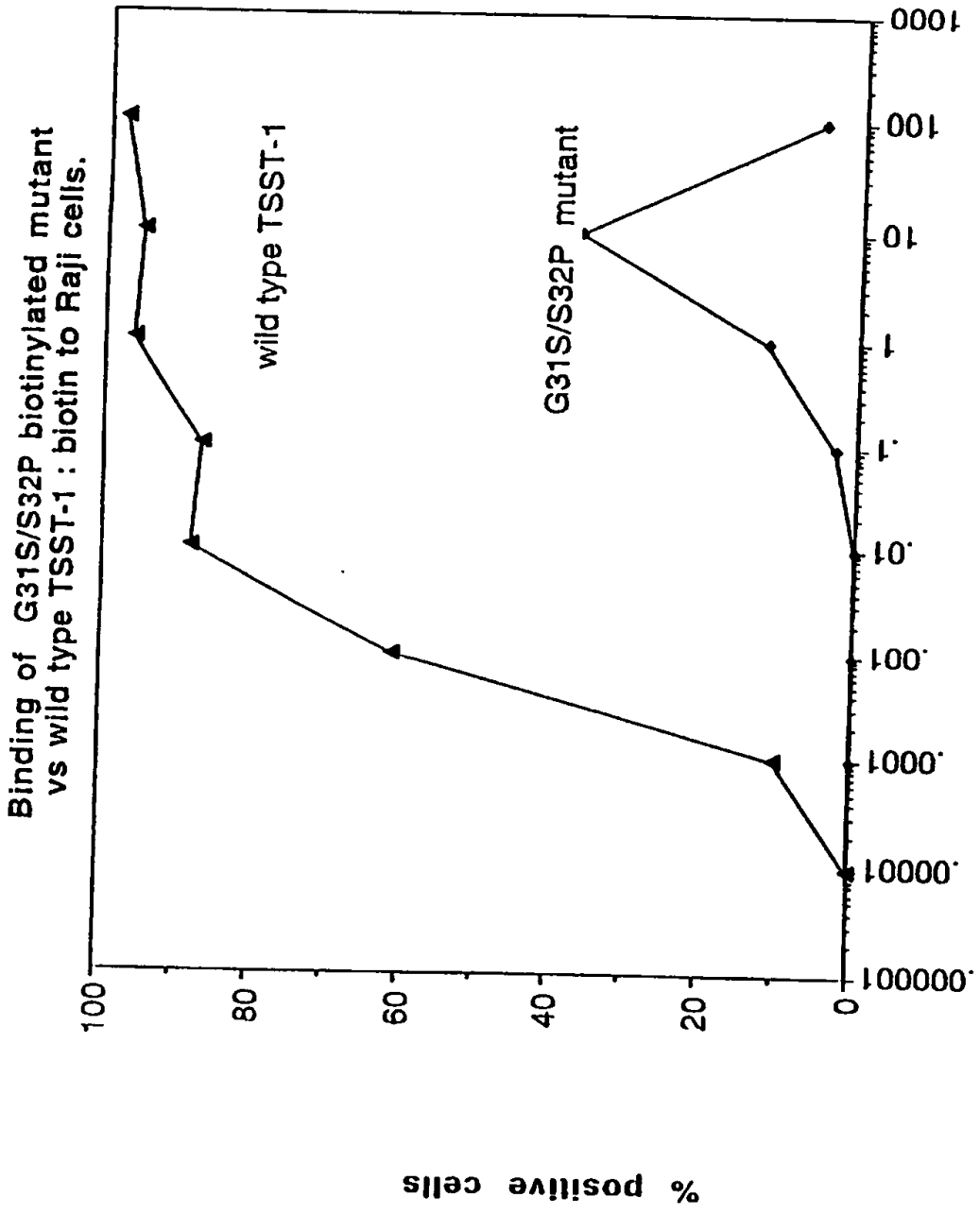
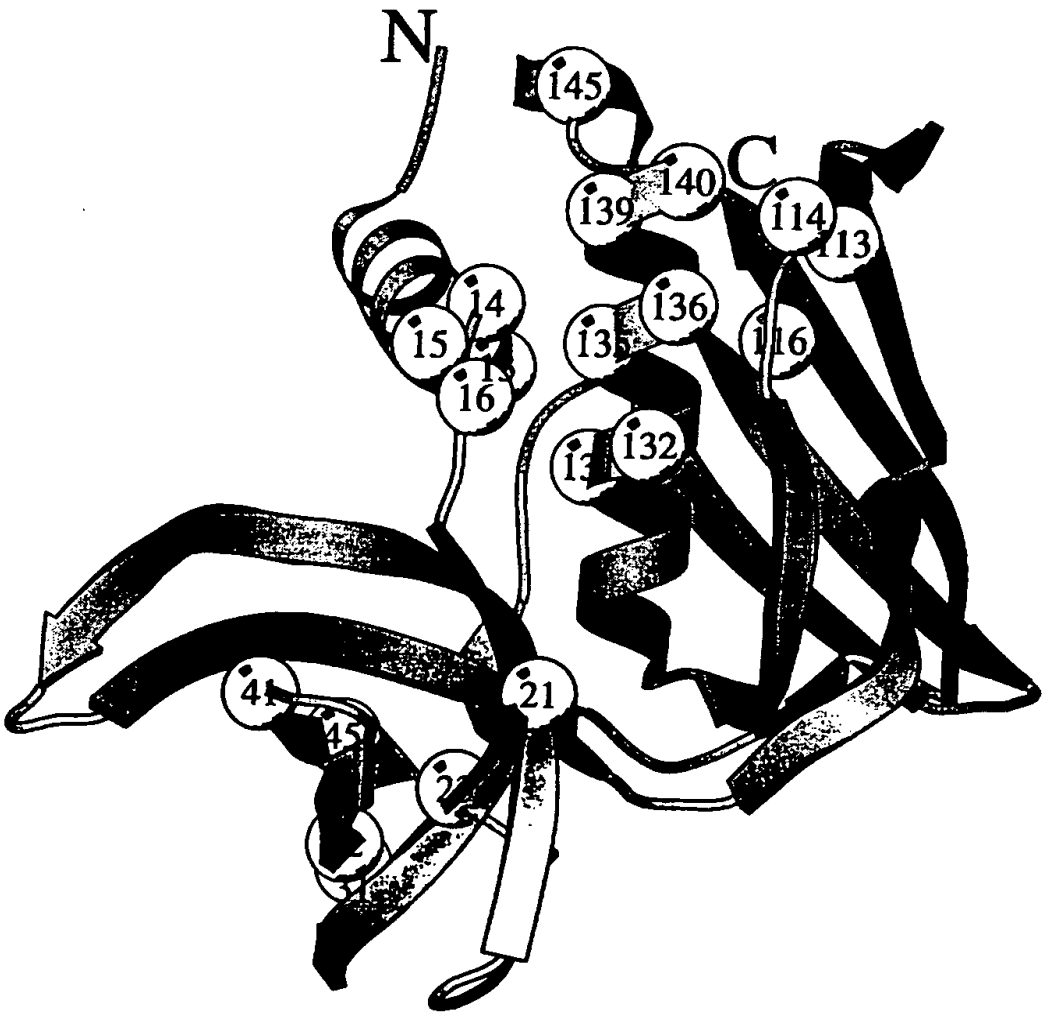

Binding of G31S/S32P biotinylated mutant
vs wild type TSST-1 : biotin to Raji cells.
wild type TSST-1
G31S/S32P mutant
% positive cells
100
80
60
40
20
0
1000
100
10
1
.1
.01
.001
.0001
.00001
.000001
ug/ml mutant vs wild type protein

FIG. 5B



N-terminal Domain C-terminal Domain

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14639

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :424/ 237.1, 243.1; 514/ 2; 530/ 350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/ 237.1, 243.1; 514/ 2; 530/ 350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A, 93/14634 (KAPPLER ET AL) 05 August 1993, see entire document.	1-12, 15-19
X	WO, A, 94/22474 (KOTZIN ET AL) 13 October 1994, see entire document.	1-25
X	Infection and Immunity, Volume 58, No. 9, issued September 1990, Blanco et al, "Mutants of Staphylococcal Toxic Shock Syndrome Toxin-1: Mitogenicity and Recognition by a Neutralizing Monoclonal Antibody", pages 3020-3028, see at least pages 3022, 3023, 3026.	16-25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 FEBRUARY 1996	01 MAR 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer H. F. Sidberry Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14639

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Experimental Medicine, Volume 175, issued 1992, Kappler et al, "Mutations Defining Functional Regions of the Superantigen Staphylococcal Eneterotoxin B", pages 387-396, see pages 391-393.	1-13, 15-19, 16-18
X	WO, A, 91/04053 (KALLAND ET AL.) 04 April 1991, see Abstract, pages 11.	1-12, 15-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14639

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01N 37/18; A61K 37/00, 39/02; C07K 1/00, 14/00, 17/00; C12P 21/04, 21/06; C12N 5/00, 15/00